CHARGE NO.	6906
PROJECT TITLE:	BIOLOGICAL EFFECTS OF SMOKE
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I. *SALMONELLA*/MICROSOME (S/M) MUTATION ASSAY--CHEMICAL DETERMINANTS OF IT CSC ACTIVITY (with 6908)

One of the continuing goals of the collaborative studies with Project 6908 has been to isolate and identify certain compounds which are responsible for impaction trap cigarette smoke condensate (IT CSC) activity. Two compounds, MW 270 and MW 211, which were first seen in the burley base fraction and then later reisolated by reverse phase liquid chromatography (RPLC) from LH-20 fraction 8, were tested using strain TA98 with metabolic activation (+S9). Also tested were LH-20 fraction 7 and its six RPLC subfractions.[1] The results indicated that MW 270 and MW 211 were both active. LH-20 fraction 7 and each of its six subfractions also showed activity.

II. S/M ASSAY (STRAIN TA98)--ASSAY RESPONSE USING A MODIFIED FORWARD MUTATION ASSAY PROTOCOL

Previous experiments to include a measure of toxicity as part of the Ames *Salmonella typhimurium* assay, using the protocol of the 8-azaguanine forward mutation assay, were not successful. A modified version of this protocol was then designed and tested with 2R1 CSC.[2] In a similar manner as the forward mutation protocol, the modified protocol was designed to quantitatively test for both the number of revertants (mutation) and the number of survivors (toxicity) using the same exposed cell population. The effect of different incubation times (0, 30, 60, and 120 minutes) for the reaction mixtures were tested at each 2R1 dose. Aside from trying to mimic the two hour incubation time used for the 8-azaguanine assay, the purpose for testing these various incubation times was to determine if the incubation time would affect the observed response using TA98.

The data indicate that the number of revertants increased at all doses as the incubation time increased. This increase was most evident for the one and two hour incubation times, along with evidence of toxicity especially at the CSC dose of 1.0 mg. Using the toxicity data, the mutation frequency (number of revertants per exposed cell population) was calculated. Future studies will be conducted to determine what type of toxicity determination, if any, could be included as a modification to the Ames assay protocol.

III. S/M ASSAY--SIDESTREAM AND MAINSTREAM SMOKE STUDIES (with 6910)

In our continuing studies with sidestream (SS) and mainstream (MS) CSC, the members of Project 6910 submitted duplicate SS and MS samples from five model cigarette types collected from an European design smoke collection chamber.[3] All

samples were dry impaction trap (DIT) collected and tested in strains TA98 and TA100 (+S9). An additional sample tested represented the residue left on the walls of the chamber after 2R1 SS collection.

The activities from all five model cigarette types indicated that MS CSCs were more active than the SS CSCs in both strains. All of the MS samples were more active in TA98 than in TA100. The activity of the 2R1 chamber SS sample was half the activity of the 2R1 IT SS counterpart.

The January progress report contained activity information on a set of 2R1 MS and SS fractions collected by several procedures.[4,5] The results were difficult to interpret due to the unexpectedly high specific activity of the 2R1 SS sample. After analysis of the samples for water and nicotine, an accidental interchange of two samples was discovered and corrected. The 2R1 IT SS and an electrostatic precipitation collected SS sample had been mislabeled. The corrected results will be included in forthcoming 6910 and 6906 Annual Reports. As expected, the correctly identified 2R1 IT SS sample is now less active than the MS in both TA98 and TA100, which is in agreement with previous results.[6]

IV. REFERENCES

1. Burke, B. K.; Tickle, M. H. PM Notebook No. 7750/32, pp. 184-190.
2. Burke, B. K.; Tickle, M. H. PM Notebook No. 7750/31, pp. 176-183.
3. McCoy, W. R.; Thompson, L. H. PM Notebook No. 7762/6-7, pp. 82-92; 93-104.
4. Thompson, L. H. Monthly Progress Report. Monthly progress report 83-013; 1983 February 15.
5. McCoy, W. R.; Thompson, L. H. PM Notebook No. 7762, pp. 80-81.
6. Burke, B. K. Monthly Progress Report. Monthly progress report 82-292; 1982 December 15.

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